UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

November 8, 2012

MEMORANDUM

Subject: Acute Toxicity Review for EPA File Symbol 75340-A
Data Package 404722
Product Name: ORD-X209

From: Wallace Powell, Biologist
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Product Science Branch
Antimicrobials Division (7510P)

Through: Karen Hicks, Team Leader
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To: Jacqueline Campbell, PM 34/ Jaclyn Carl
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Osmose Railroad Services, Inc.

FORMULATION FROM PROPOSED LABEL:

Active Ingredients:	% by weight
Copper ethanolamine complex	5.84
Disodium octaborate tetrahydrate	5.00
Other Ingredient(s):	72.89
Total:	100.00

BACKGROUND

In support of registration of the subject product ORD-X209, EPA File Symbol 75340-A, the applicant has submitted studies for acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, eye irritation, skin irritation, and skin sensitization – MRIDs 488852-03 through -08, respectively.

PSF check WP 11/9/12

RECOMMENDATION

The submitted studies are acceptable. The resulting acute Toxicity Categories are listed in the table below.

Summary:

The acute toxicity profile of ORD-X209 is currently:

Study	MRID	Toxicity Category	Status
Acute Oral Toxicity	488852-03	III	Acceptable
Acute Dermal Toxicity	488852-04	IV	Acceptable
Acute Inhalation Toxicity	488852-05	IV	Acceptable
Primary Eye Irritation	488852-06	III	Acceptable
Primary Skin Irritation	488852-07	II	Acceptable
Dermal Sensitization	488852-08	Sensitizer	Acceptable

PRODUCT LABELING

The following comments refer to the proposed draft label dated by applicant "JUN-2012" (EPA received 7/13/2012).

Revisions:

The following changes should be made to the "Hazards to Humans and Domestic Animals" statements, in accordance with the *Label Review Manual*:

1. Add the statement: "Causes skin irritation."

2. Change the skin portion of the statement "Avoid contact with skin, eyes or clothing" so that it reads: "Do not get on skin or on clothing. Avoid contact with eyes." (An alternative would be: "Do not get in eyes, on skin or clothing.")

The following statements should be added to the First Aid section:

If inhaled:

Move person to fresh air.

If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.

Call a poison control center or doctor for further treatment advice.

In the First Aid section, the "If on skin" instructions should be listed first. (The *Label Review Manual* states: "First Aid statements should be organized so that the most severe routes of exposure, as demonstrated by the toxicity classification, are listed first.")

Other comments:

The statements for Personal Protective Equipment and for User Safety appear acceptable in light of existing Reregistration Eligibility Decision (RED) documents (the *Boric Acid* and *Coppers* REDs).

The Reregistration Eligibility Decision (RED) for *Coppers* contains a "General Application Restrictions for products with WPS or non-WPS uses on the label," to be placed in the Directions for Use (following the misuse statement). The Agency Product Manager may determine whether that statement is applicable and required:

> "Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State or Tribe, consult the State or Tribal agency responsible for pesticide regulation."

Information regarding the product (ORD-X209) has been entered into the Label Review System (LRS). The resulting sample labeling for the product in LRS is available for internal reference.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 34
MRID No.: 488852-03

Reviewer: W. Powell
Study Completion Date: 4/11/2012
Report No.: 33780

Testing Laboratory: Product Safety Labs – Dayton, NJ
Author: Aija McKenzie

Quality Assurance (40 CFR §160): Included

Test Material: ORD-X209. (Shaken prior to dosing)
Dosage: 175, 550, and 2,000 mg/kg

Species: Rat, Sprague-Dawley derived
Sex: 8 Females (nulliparous and non-pregnant)
Age: 8-11 weeks
Weight: 164-203 g
Source: Harlan Laboratories, Inc.

Method: Up-and-Down Procedure

Summary:

1. **Estimated LD_{50}**: 2,000 mg/kg
2. **Toxicity Category**: III
3. **Classification**: Acceptable

Deviations from Guideline 870.1100: None noted.

Results:

The test substance was administered by oral gavage to eight female rats, in the sequence listed in the table below. Three consecutive animals survived at the upper bound, thus a stopping rule was met. The one decedent animal died within one day of test suhstance administration.

Based on the *AOT425* software (Westat, May 2001), the results indicate an acute oral LD_{50} of 2,000 mg/kg. 95% PL Confidence Interval: 1,590 mg/kg to greater than 20,000 mg/kg.

Reported Mortality

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	175	S	S
2	3102	550	S	S
3	3103	2,000	S	S
4	3104	2,000	D	D
5	3105	550	S	S
6	3106	2,000	S	S
7	3107	2,000	S	S
8	3108	2,000	S	S

S = Survival, D = Death

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 34
MRID No.: 488852-04

Reviewer: W. Powell
Study Completion Date: 4/11/2012
Report No.: 33781

Testing Laboratory: Product Safety Labs – Dayton, NJ
Author: Aija McKenzie

Quality Assurance (40 CFR §160): Included

Test Material: ORD-X209. (Applied as received)
Dosage: 5,000 mg/kg

Species: Rat, Sprague-Dawley derived
Sex: 5 Males, 5 Females (nulliparous and non-pregnant)
Age: 9-10 weeks
Weight: Males 241-268 g, Females 180-201 g
Source: Harlan Laboratories, Inc.

Summary:

1. **Estimated LD_{50}**: > 5,000 mg/kg
2. **Toxicity Category**: IV
3. **Classification**: Acceptable

Deviations from Guideline 870.1200: None noted.

Results:

Administration of the test substance by dermal application at a dose of 5,000 mg per kg body weight to male and female rats produced no mortality during the 14-day observation period. The results indicate that the dermal LD_{50} of the sample was greater than 5,000 mg/kg in male and female rats. Clinical signs included dermal erythema, blanching, and/or small areas of eschar, at the dose site, in all ten animals. Necropsy revealed no gross abnormalities. All animals showed weekly weight gain.

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Combined
5,000	0 / 5	0 / 5	0 / 10

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)

Product Manager: 34
MRID No.: 488852-05

Reviewer: W. Powell
Study Completion Date: 4/11/2012
Report No.: 33782

Testing Laboratory: Product Safety Labs – Dayton, NJ
Author: Aija McKenzie

Quality Assurance (40 CFR §160): Included

Test Material: ORD-X209. (Shaken prior to dispensing.)
Concentration: Gravimetric = 2.08 mg/L, Nominal = 39.62 mg/L. (Nose-only exposure)

Species: Rat, Sprague-Dawley derived
Sex: 5 Males, 5 Females (nulliparous and non-pregnant)
Age: 11-12 weeks
Weight: Males 279-318 g, Females 197-223 g
Source: Harlan Laboratories, Inc.

Summary:

1. **LC_{50} (mg/L)**: > 2.08 mg/L
2. **Average MMAD**: 1.88 µm
3. **Toxicity Category**: IV
4. **Classification**: Acceptable

Deviations from Guideline 870.1300: It appears that the total volume of the animals was greater than 5% of the chamber volume. However, in view of the plentiful airflow rate, this is not a concern.

Results:

All animals survived the 4-hour exposure. The median lethal concentration is therefore estimated to be greater than 2.08 mg/L in male and female rats.

Following exposure, all animals exhibited irregular respiration but recovered by Day 8. All animals gained weight during the study, though all animals lost weight by Day 1 and two females failed to gain weight between Days 3 and 7. Necropsy revealed no gross abnormalities.

Reported Mortality

Exposure Concentration (mg/L)	Number of deaths / number tested		
	Males	Females	Combined
2.08	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exposure Conc. (mg/L)	MMAD (µm)	GSD (µm)	% of Particles ≤ 4.7 µm
2.08	1.88	2.24	87.8

Chamber Environment

Exposure Level (mg/L)	2.08
Chamber Volume, approx. (L)	28
Total Airflow Rate (Lpm)	31.6
Temperature in exposure tube (°C)	20 - 21
Relative Humidity in exposure tube (%)	31 - 35

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 34
MRID No.: 488852-06

Reviewer: W. Powell
Study Completion Date: 4/11/2012
Report No.: 33783

Testing Laboratory: Product Safety Labs – Dayton, NJ
Author: Aija McKenzie

Quality Assurance (40 CFR §160): Included

Test Material: ORD-X209. (Shaken prior to dosing.)
Dosage: 0.1 mL

Species: Rabbit, New Zealand albino
Sex: 3 females (nulliparous and non-pregnant)
Age: Young adult
Weight: Not reported
Source: Robinson Services, Inc.

Summary:

1. **Toxicity Category**: III
2. **Classification**: Acceptable

Deviations from Guideline 870.2400: The study report did not state that the lids of the treated eye were held together for about 1 second following test substance instillation. (However, Product Safety Labs' Carolyn Lowe told the reviewer on 10/22/2012 that this procedural step is always taken.)

Results:

No corneal opacity or iridal involvement were observed during the 7-day observation period. 'Positive' conjunctival effects were limited to grade 2 on the Draize scale and were observed in 2/3 animals at 1 hour and in 1/3 at hours 24 through 72.

Incidence of Irritation

Time Post-Instillation	No. of Animals Testing "Positive" / No. of Animals Tested			
	Corneal Opacity	Iritis	Conjunctiva	
			Redness	Chemosis
1 hour	0 / 3	0 / 3	2 / 3	1 / 3
24 hours	0 / 3	0 / 3	1 / 3	0 / 3
48 hours	0 / 3	0 / 3	1 / 3	0 / 3
72 hours	0 / 3	0 / 3	1 / 3	0 / 3
Day 4	0 / 3	0 / 3	0 / 3	0 / 3
Day 7	0 / 3	0 / 3	0 / 3	0 / 3

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 34
MRID No.: 488852-07

Reviewer: W. Powell
Study Completion Date: 4/11/2012
Report No.: 33784

Testing Laboratory: Product Safety Labs – Dayton, NJ
Author: Aija McKenzie

Quality Assurance (40 CFR §160): Included

Test Material: ORD-X209. (Shaken prior to dosing)
Dosage: 0.5 mL

Species: Rabbit, New Zealand albino
Sex: 3 females (nulliparous and non-pregnant)
Age: Young adult
Weight: Not reported
Source: Robinson Services, Inc.

Summary:

1. **Toxicity Category**: II
2. **Classification**: Acceptable

Deviations from Guideline 870.2500: None noted.

Results:

Two of three animals showed very slight erythema. Erythema in one animal was severe from 24 hours through Day 10. This animal showed a small area of eschar in the dose site from 24 hours through Day 10, slight edema from 24 hours through 72 hours, and desquamation at the dose site on Days 7 through 14.

The Guidelines define the skin irritation Toxicity Category in terms of the effects at 72 hours. In view of that, and in view of the small number of animals tested, and the severity of response in animal 3503 and its lack of improvement until late in the study, a strong emphasis is being placed on animal 3503. The severe irritation in that animal cleared after Day 10. In view of all this, Toxicity Category II is assigned (even though the study report states that the requirements of Category III were met, a conclusion that may have been based in large part on the Primary Irritation Index).

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema						
		Time After Patch Removal						
		30-60 min	24 hrs	48 hrs	72 hrs	Day 7	Day 10	Day 14
3501	F	1 / 1 [1]	0 / 1 [1]	0 / 0 [1]	0 / 0 [1]	0 / 0 [1]	0 / 0 [1]	0 / 0
3502	F	1 / 0	2 / 1	1 / 0	1 / 0	0 / 0 [2]	0 / 0 [2]	0 / 0 [2]
3503	F	1 / 1 [3]	4 / 2 [4]	4 / 2 [4]	4 / 2 [4]	4 / 1 [2,4]	4 / 1 [2,4]	0 / 0 [2]

[1] Faint blue staining in the dose site
[2] Desquamation present at the dose site
[3] Blue discoloration in the dose site
[4] Small area of eschar in the dose site

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)

Product Manager: 34
MRID No.: 488852-08

Reviewer: W. Powell
Study Completion Date: 4/11/2012
Report No.: 33785

Testing Laboratory: Product Safety Labs – Dayton, NJ
Author: Aija McKenzie

Quality Assurance (40 CFR §160): Included

Test Material: ORD-X209 (a dark blue liquid; shaken well prior to dosing)
Positive Control Material: alpha-Hexylcinnamaldehyde

Species: Mouse, CBA/J, Female
Weight: 18.7-23.3 g (Test and Control groups)
Age: 10-11 weeks (Test and Control groups), 9-10 weeks (Preliminary animals)
Source: Harlan Laboratories, Inc.

Method: Local Lymph Node Assay

Summary:

1. ORD-X209 appeared to be a contact sensitizer.
2. **Classification:** Acceptable

Deviations from Guideline 870.2600:

- Temperature in the animal room slightly exceeded the Guidelines.
- Based on body weights reported, it appears that the Vehicle Control and Positive Control animals were the same as those used in Product Safety Labs study 33791 (MRID 488846-08, reviewed in Data Package 403967). Presumably this is because (as appears to be the case) the two studies were conducted concurrently and the Vehicle Control and Positive Control substances appear appropriate for both studies. Thus the sharing of these animals between the two studies was appropriate.

Procedure:

Five female mice were selected for one Test Substance group. (No further Test Substance groups were treated, presumably because this group showed a positive response.) On Days 1, 2, and 3, 25 µL of a 25% concentration of the test substance in propylene glycol was applied to the dorsum of both ears of each test animal. A Vehicle Control group (5 females) was treated in the same way as the Test Substance group, but with vehicle alone (propylene glycol). A Positive Control group (5 females) was treated with 25% alpha-hexylcinnamaldehyde in propylene glycol. The test sites in all three groups were evaluated for local irritation (erythema & edema) on Days 1, 2, 3, and 6. Body weights were taken on Days 1 and 6.

On Day 6, the animals in all three groups were injected in the tail vein with 250 µL of phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-methyl thymidine. Approximately five hours after the injection, the draining auricular lymph nodes were excised and pooled for each mouse.

A single cell suspension of lymph node cells was prepared in PBS by massaging the lymph nodes between frosted ends of two microscope slides over a collection vessel. The contents of the vessel were transferred to a centrifuge tube, washed with an excess of PBS, and centrifuged for approximately 10 minutes at 1800 rpm. This wash and centrifuge was carried out twice. The supernatant was decanted and discarded following each centrifugation. After the second wash, approximately 5 mL of 5% trichloroacetic acid (TCA) was added to the sediment and the tube was briefly vortexed. The DNA was then precipitated in the TCA at approximately 4.1-4.2 °C for approximately 18 hours. The tubes were centrifuged again. The resulting precipitate was re-suspended in TCA and transferred to 10 mL of scintillation fluid. Incorporation of ^{3}H-methyl thymidine was measured by B-scintillation counting and expressed as disintegrations per minute (DPM) minus background DPM.

Results:

Stimulation Index (SI) for Test Substance group and for Positive Control group was derived by dividing the average net DPM of each group by the average net DPM of the Vehicle Control group, where "net DPM" is the measured DPM minus the background DPM.

Animal Group	Test Substance Concentration	Average Net DPM	Number of Mice	SI
Vehicle Control	N/A	808.99	5	N/A
Positive Control	N/A	4032.46	5	4.98
Test Substance	25%	4651.82	5	5.75

An SI well in excess of 3.0 was observed in the Test Substance group, thus indicating that ORD-X209 tested positive for dermal sensitization. Note: No signs of dermal irritation were observed in the Test Substance group (nor in the Vehicle Control group). The Positive Control group tested positive.